PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IT2004/000689	10.12.2004	11.12.2003

International Patent Classification (IPC) or both national classification and IPC
A61K31/4985, A61K31/505, A61K31/475, A61K38/12, A61K38/16, G01N33/574, C07K7/00, A61P35/04

Applicant
TIGEM

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IT2004/000689

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 17-28

because:

☒ the said international application, or the said claims Nos. 17-28 with regard to industrial application relate to the following subject matter which does not require an international preliminary examination *(specify)*:

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the whole application or for said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	12,13,39,42
	No:	Claims	1,11,17,29,40,41
Inventive step (IS)	Yes:	Claims	12
	No:	Claims	1-11,13-46
Industrial applicability (IA)	Yes:	Claims	1-16,29-42
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 17-28 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2. Prior art:

Reference is made to the following documents:

D1: US-B1-6 486 300 (BANDMAN OLGA ET AL) 26 November 2002 (2002-11-26)

D2: COLLIER G R ET AL: "INHIBITION OF LUNG METASTASIS FORMATION BY A RAT OSTEOGENIC SARCOMA SUBCLONE USING PYRIMIDO-PYRIMIDINE DERIVATIVES" AUSTRALIAN AND NEW ZEALAND JOURNAL OF MEDICINE, ROYAL AUSTRALASIAN COLLEGE OF PHYSICIANS, SYDNEY, AU, vol. 15, no. 1, SUPPL 1, February 1985 (1985-02), page 127, XP008046052 ISSN: 0004-8291

D3: BANDO H ET AL: "EFFECTS OF ANTIPLATELET AGENTS ON PULMONARY METASTASES" GANN, JAPANESE CANCER ASSOCIATION, TOKYO, JP, vol. 75, no. 3, March 1984 (1984-03), pages 284-291, XP009013087 ISSN: 0016-450X

D4: BERTRAM J S ET AL: "INHIBITION OF NEOPLASTIC CELL GROWTH BY QUIESCENT CELLS IS MEDIATED BY SERUM CONCENTRATION AND CYCLIC AMP PHOSPHO DI ESTERASE INHIBITORS" JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 18, no. 4, 1982, pages 515-538, XP002329792 ISSN: 0730-2312

D5: NI XIAOHUA ET AL: "Isolation and characterization of a novel human NM23-

H1B gene, a different transcript of NM23-H1." JOURNAL OF HUMAN GENETICS, vol. 48, no. 2, February 2003 (2003-02), pages 96-100, XP002329793 ISSN: 1434-5161

D6: POSTEL EDITH H ET AL: "Mutational analysis of NM23-H2/NDP kinase identifies the structural domains critical to recognition of a c-myc regulatory element" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 93, no. 14, 1996, pages 6892-6897, XP002329794 ISSN: 0027-8424

D7: REYMOND ALEXANDRE ET AL: "Evidence for interaction between human PRUNE and nm23-H1 NDPKinase" ONCOGENE, vol. 18, no. 51, 2 December 1999 (1999-12-02), pages 7244-7252, XP002329795 ISSN: 0950-9232

D8: FORUS ANNE ET AL: "Amplification and overexpression of PRUNE in human sarcomas and breast carcinomas: A possible mechanism for altering the nm23-H1 activity" ONCOGENE, vol. 20, no. 47, 18 October 2001 (2001-10-18), pages 6881-6890, XP002329796 ISSN: 0950-9232

D9: ZOLLO M ET AL: "Prune and nm23-H1 and nm-23 H2 (NDP-Kinase) proteins: Involvement in cancer" AMERICAN JOURNAL OF HUMAN GENETICS, vol. 69, no. 4 Supplement, October 2001 (2001-10), page 273, XP009047948 & 51ST ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS; SAN DIEGO, CALIFORNIA, USA; OCTOBER 12-16, 2001 ISSN: 0002-9297

D10: DATABASE Geneseq [Online] 26 June 2001 (2001-06-26), "Human cDNA clone (5'-primer) SEQ ID NO:5290." XP002329797 retrieved from EBI accession no. GSN:AAH08455 Database accession no. AAH08455

D11: DATABASE Geneseq [Online] 6 November 2003 (2003-11-06), "Human intracellular signalling molecule INTSIG-44, SEQ ID NO:44." XP002329798 retrieved from EBI accession no. GSN:ADA13362 Database accession no. ADA13362

D12: DANGELO A ET AL: "The human cyclic nucleotides phosphodiesterase (PDE) Prune protein: A dual cellular compartment localization and functional properties." AMERICAN JOURNAL OF HUMAN GENETICS, vol. 71, no. 4 Supplement, October 2002 (2002-10), page 513, XP002329885 & 52ND ANNUAL MEETING OF THE AMERICAN SOCIETY OF HUMAN GENETICS; BALTIMORE, MD, USA; OCTOBER 15-19, 2002 ISSN: 0002-9297

If not indicated otherwise the relevant passages are those mentioned in the search report.

Document D1 discloses the use of human nm23, comprising a peptide sequence of SEQ ID No 9 of the application, for inhibiting metastasis.

Document D2 discloses the use dipyridamole for inhibiting metastasis.

Document D3 discloses the inhibition of metastasis by dipyridamole

Document D4 discloses the inhibition of metastasis of Lewis lung carcinoma by the PDE inhibitor isobutyl-methylxanthine.

Document D5 discloses the sequence of nm23-H2, defined as a putative metastasis suppressor, which comprises a peptide of sequence of SEQ ID No 9 of the application.

Document D6 discloses the sequence of nm23, comprising an amino acid sequence of SEQ ID No 9 of the application, which is a presumed regulator of tumour metastasis.

Document D7 discloses that Prune interacts with nm23 and the uncoupling of this interaction might lead to neuroblastoma progression.

Document D8 discloses the over-expression and amplification of PRUNE assessed by immunohistochemistry, FISH and northern blot in tumours expressing nm23 and in metastasising tumours.

Document D9 discloses the interaction of the PDE Prune with the tumour metastasis inhibitor gene nm23-H1. Document D9 discloses that Prune is amplified in tumour cells as shown by FISH and immunohistochemistry.

Document D10 discloses a nucleic acid sequence comprising the sequence of SEQ

ID No 1 of the application which is 5'-primer.

Document D11 discloses a peptide comprising a sequence of SEQ ID No 4 of the application and antibodies specific for this peptide.

Document D12 discloses that the Prune protein possesses phosphodiesterase activity.

2. Novelty (Art. 33 (1) and (2) PCT):

2.1 Claim 1 is not novel over the disclosure of documents D1-D4. These documents do not disclose that nm23, dipyridamole, or IBMX are inhibitors of the cyclic nucleotide phosphodiesterase activity of Prune, however documents D1-D4 disclose the inhibition of metastasis by these compounds. The presence of a mechanism of action described in the application, i.e inhibition of Prune activity, cannot be used to delimit the present claims from the state of the art. The end effect of the presently claimed invention is the treatment of metastasis using the same compounds as disclosed in the prior art. The mechanism of action is therefore merely a discovery of how the compounds according to the prior art could work. Claim 1 does not fulfill the requirements of Art. 33(2) PCT.

2.2 Claim 11 is not novel over documents D1 and D5-D6 which disclose peptides comprising a sequence of SED ID No 9. Claim 11 does not fulfill the requirements of Art. 33(2) PCT.

2.3 Claims 17 lacks novelty over document D8-D9 which discloses the increased expression of Prune in metastasising tumours (see p6882 col2 1st §). Claim 17 does not fulfill the requirements of Art. 33(2) PCT.

2.4 Claim 29 lacks novelty over documents D7-D8.

2.5 Claim 40 lacks novelty over document D11.

2.6 Claim 41 is not novel over document D10.

3. Inventive step (Art. 33 (1) and (3) PCT):

3.1 The peptide of claim 12 is neither disclosed nor suggested in the prior art. Claim 12 fulfills the requirement of Art. 33(3) PCT.

3.2 Document D12 discloses that the Prune protein possesses phosphodiesterase catalytic activity. The method of screening for inhibitors of phosphodiesterase of claim 13 would be obvious for a skilled man. Claim 13 does not fulfill the requirement of Art. 33(3) PCT.

3.3 Antibodies directed to Prune are known in the art (see D8: p6885 fig3). It would be obvious for a skilled man to produce an alternative monoclonal antibody specific for Prune. Claim 39 does thus not involve an inventive step.

3.4 The sequence of Prune is known from document D7, it would be obvious for a skilled man to provide an oligonucleotide probe specific for Prune. Claim 42 does not involve an inventive step.

3.5 Dependent claims 2-9, 14-16, 18-28, 30-38 and 43-46 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step.

Re Item VII

Certain defects in the international application

4. It seems that there is a typographic error in the sequences of SED ID 9 and 10 of claims 8-12.